In vitro propagation of *Lapeirousia silenoides*

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A successful means of *in vitro* propagation of *Lapeirousia silenoides* (Jacq.) Ker, is described. A section of the corm containing a growing point was used as the explant. Basal medium (BM) supplemented with 1 mg dm^{-3} BAP resulted in good shoot proliferation. Proliferation continued when the shoots were subcultured on a BM containing 0.1 mg dm^{-3} NAA and 1 mg dm^{-3} BAP. Optimal root formation was obtained on a BM containing 1 mg dm^{-3} IBA. Cytokinins delayed corm development. The *in vitro* plantlets could be forced to develop corms by keeping them on the medium for 8 to 16 weeks. Transplantation of *in vitro* rooted plants into soil was 100% successful.

Die suksesvolle *in vitro*-vermeerdering van *Lapeirousia silenoides* (Jacq.) Ker., word beskryf. Die groeipunt tesame met 'n deel van die knol is as eksplant gebruik. Basale medium (BM) met 1 mg dm^{-3} BAP het lootproliferasie veroorsaak. Die proliferasie het voortgeduur indien die *in vitro*-lote op 'n BM met 0.1 mg dm^{-3} NAA en 1 mg dm^{-3} BAP gesubkultuur is. Wortels is verkry op 'n BM met 1 mg dm^{-3} IBA. Sitokiniene het *in vitro*-knolontwikkeling vertraag. Knolvorming kan by die *in vitro*-plantjies geïnduseer word, deur hulle vir 8 tot 16 weke op die medium te laat. 'n Honderd persent oorlewing is verkry met uitplanting in grond.

Keywords: *In vitro* propagation, *Lapeirousia silenoides*, tissue culture

Introduction

Lapeirousia silenoides (Jacq.) Ker. belonging to the Iridaceae family, is common throughout Namaqualand. The plant sometimes forms a bunch of small cerise flowers which makes a beautiful display (Figure 1) and it was this feature which first attracted attention to this small plant. If multiple flowering could be induced, *L. silenoides* could be used as a beautiful pot plant. In order to study the factors involved in the production of multiple flowers, a sufficient number of corms are necessary. Since *L. silenoides* is slow to multiply vegetatively and *in vitro* multiplication of other cormous species has already been shown to be very successful (Hussey 1977; Sutter 1986; Ziv *et al.* 1970), it was decided that an *in vitro* technique should be developed for the multiplication of the corms. This technique could also be used to commercialize the species.

Materials and Methods

Plant material

Mature *L. silenoides* plants were collected in the western Cape during their flowering period. Two weeks later the corms were cultured. At the time of culture, leaf and flower senescence had already taken place.

Surface sterilization and culture procedure

The outer paper-like tunic was peeled off the corms before sterilization. The corms were sterilized by shaking for 20 min in 5% '7X' solution (manufactured for Seravac Division Fine Chemical Corporation), followed by 1 min in 70% ethanol and 20 min in 2% sodium hypochlorite (NaOCl). The material was subsequently rinsed twice in sterile, distilled water. After 2 weeks in culture, 85% of the explants showed signs of contamination. They were resterilized by shaking for 20 min in 2% sodium hypochlorite followed by rinsing three times in sterile, distilled water. The explants were dipped in 0.2% sodium hypochlorite for 10 sec, before being placed on the culture medium. After this resterilization only 3% of the explants were contaminated.

The corms were cut in half transversely and placed with the cut surface on the nutrient medium. The basal culture medium (BM) consisted of the following: the salt mixture described by Murashige & Skoog (1962) with the macro-elements at half the normal concentration, NaFeEDTA (25 mg dm^{-3}), myo-inositol (100 mg dm^{-3}), thiamine-HCl (0.5 mg dm^{-3}), sucrose (30 g dm^{-3}) and agar (7 g dm^{-3}). The pH was adjusted to 5,8 by using KOH. Ten ml of medium was dispensed into 19 × 150 mm test tubes and autoclaved at 121°C for 20 min. At least 25 replicates were used per treatment. All cultures were maintained at 25°C (± 3°C). Light was provided at an intensity of 4 000 lux by cool white fluorescent tubes for 16 h per day.

Random designs were used in all experiments and differences between treatments were detected by the Tukey test at a 95% confidence level (Statgraphics 1986).

Initiation of shoot proliferation

The initial culture medium consisted of the BM supplemented with various concentrations of 1-naphthalene acetic acid (NAA) and/or 6-benzylaminopurine (BAP). Results were taken after a 6-week culture period.

Maintenance of shoot proliferation

In order to find an appropriate medium for further proliferation the shoots from cultures on the three best initiation media were each subcultured on these three media for a further 6 weeks. Each combination of initiation and subculture medium was considered as a treatment.

Figure 1 *Lapeirousia silenoides* (Jacq.) Ker.

Root and corm development

Shoots produced *in vitro* were used to investigate the effect of IBA and BAP levels on root and corm development. Results were taken after 8- and 16-week culture periods.

Transplantation of plants produced *in vitro*

Rooted plantlets were planted into small plastic pots containing a soil mixture consisting of 1:1:1 peat, soil and bark. These plants were placed next to the wet wall of a glasshouse until they became dormant.

Results and Discussion

Unlike the axillary buds of most corms, those of *L. silenoides* are not distributed on the corm but are centered around the apical bud. The only shoot proliferation therefore took place at the growing point of the corm. Some of these shoots developed lateral shoots, which were also counted as shoots, as proliferation of these shoots did occur when subcultured. The effect of different hormone combinations on initiation of shoot proliferation is summarized in Table 1. Most of the treatments were not significantly different at the 5% level.

The results of the shoot proliferation experiments are summarized in Table 2. There were no significant differences between treatments where 5 mg dm^{-3} BAP were used either in initiation and/or subculture mediums. The results suggest that 5 mg dm^{-3} BAP does enhance shoot

Table 1 The mean number of *L. silenoides* shoots per explant cultured for 6 weeks on a Murashige and Skoog BM supplemented with NAA and/or BAP[a]

NAA concentration	BAP concentration (mg dm^{-3})		
(mg dm^{-3})	0.1	1	5
0	3.1A	6.3B	5.7AB
0.1	3.5A	5.6AB	4A

[a]Values not followed by the same letter are significantly different at the 5% level

Table 2 The mean number of *L. silenoides* shoots produced from *in vitro* derived shoots when subcultured on a Murashige and Skoog BM supplemented with NAA and/or BAP[a]

Treatment	Hormone conc. of initation medium (mg dm^{-3})	Hormone conc. of of subculture medium (mg dm^{-3})	Mean no. of shoots
1	NAA 0; BAP 1	NAA 0; BAP 1	10.8 A
2	NAA 0; BAP 1	NAA 0; BAP 5	14.5 AB
3	NAA 0; BAP 1	NAA 0.1; BAP 1	17.2 AB
4	NAA 0; BAP 5	NAA 0; BAP 1	12.4 AB
5	NAA 0; BAP 5	NAA 0; BAP 5	21.1 B
6	NAA 0; BAP 5	NAA 0,1; BAP 1	11.8 AB
7	NAA 0,1; BAP 1	NAA 0; BAP 1	7.2 A
8	NAA 0,1; BAP 1	NAA 0; BAP 5	12.6 AB
9	NAA 0.1; BAP 1	NAA 0.1; BAP 1	10.6 A

[a]Values not followed by the same letter are significantly different at the 5% level

Table 3 The effect of IBA and/or BAP or the percentage *in vitro* shoots of *L. silenoides* that developed roots and corms[a]

Treatment	IBA conc. (mg dm^{-3})	BAP conc. (mg dm^{-3})	% shoots that produced roots	% corm-producing shoots after 8 weeks	% corm-producing shoots after 16 weeks
1	0	0	30A	50AB	90A
2	0.1	0	60A	70AB	95A
3	0.5	0	65A	80A	90A
4	1	0	70A	60AB	90A
5	0	0.1	43A	31B	88A
6	0.1	0.1	50A	50AB	90A
7	0.5	0.1	55A	35B	85A

[a]Values not followed by the same letter are significantly different at the 5% level

proliferation. It can however be expected that prolonged exposure to such high levels of cytokinin will eventually suppress normal root and corm development (Hussey 1977). If this is observed in this micropropagation programme, it would be better to use treatment 3. This treatment contained 1 mg dm^{-3} BAP and did not differ significantly from those treatments containing 5 mg dm^{-3} BAP. A program where high BAP alternates with low BAP during successive culturing may even be considered.

During the subculturing period, flowers were sporadically produced *in vitro*. There was no significant correlation between this phenomenon and the composition of the medium.

Root formation occurred readily on a BM supplemented with 0.1, 0.5 or 1 mg dm^{-3} IBA although there were no significant differences between the treatments. The rooting percentage was low on media without IBA. When BAP was present in the medium, a lower rooting percentage was obtained and the roots were short and succulent (Table 3).

After 8 weeks in culture, the presence of BAP caused a significant difference between the percentage shoots that developed corms (Table 3). Shoots on a medium containing BAP had fewer corms than those on a medium without BAP. After 16 weeks in culture this difference was counterbalanced and almost 100% of the plants produced corms. Similar results were reported by Hussey (1977) for *Gladiolus*. A possible explanation for the above observations could be that corm development occurs when plants are placed under stress i.e. by a depletion of nutrients and hormones.

A 100% survival rate was obtained when the rooted *in vitro* plantlets were transplanted into soil. Theoretically therefore, following the technique described above, it should be possible to regenerate over 100 rooted plants from one *L. silenoides* corm within 18 weeks.

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